DATA EVALUATION RECORD

TRIFLUMEZOPYRIM

STUDY TYPE: SUBCHRONIC TOXICITY – DOG
(OCSPP 870.3150)

MRID 49382163

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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DATA EVALUATION RECORD[1]

STUDY TYPE: Subchronic Toxicity [oral] -Dog;
OCSPP 870.3150 [§82-1] (non-rodent); OECD 409.

PC CODE: 129210 **DP BARCODE:** D432127

TEST MATERIAL (PURITY): Triflumezopyrim (99.4% ai)

SYNONYMS: 2, 4-Dioxo-1-(5-pyrmidinylmethyl)-3-[3-(trifluoromethyl) phenyl-2H-pyrido [1, 2-α] pyrimidium, inner salt; DPX-RAB 55 Technical

CITATION: Papagiannis, C.N. (2013) DPX-RAB55 technical: Subchronic toxicity 90-day feeding study in dogs. MPI Research, Inc. 54943 North Main Street, Mattawan, Michigan 49071 U.S.A. DuPont Report No.: DuPont-34938, MPI Research Study No.: 125-164. August 22, 2013. MRID 49382163. Unpublished

SPONSOR: E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898 U.S.A.

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 49382163), triflumezopyrim (DPX-RAB55, 99.4% ai) was administered to 4 beagle dogs/sex/dose in diet at dose levels of 0, 100, 400, 1000, or 4000 ppm (mean daily intake: 0, 3.05, 12.20, 26.60, or 114.94 mg/kg bw/day, respectively, for males and 0, 2.69, 12.15, 26.87, or 131.13 mg/kg bw/day, respectively, for females).

All animals survived until their scheduled sacrifice, with the exception of one female at 4000 ppm which was euthanized *in extremis* on Day 77. The cause of death was attributed to a hemorrhage of undetermined origin. Treatment-related clinical signs included thin appearance in males and females in the 4000 ppm group. Treatment-related reductions in overall body weight and body weight gain were noted in males at 4000 ppm and in females at doses of 1000 ppm or higher, when compared to controls. These body weight changes were associated with reduced food consumption and/or food efficiency at these concentrations; however, decreases in food efficiency at 1000 ppm were minimal (1-3%). Although changes in absolute bodyweight were ≥10% at 1000 ppm, they were not considered adverse based on the weight of evidence across the available oral toxicity studies in dogs.

[1] This DER was generated by modifying the study summary in a Tier II document (MRID 49382105).

There were no treatment-related effects on coagulation or urinalysis parameters at doses up to 1000 ppm. At 4000 ppm, there were mild decreases in red cell mass accompanied by alterations in erythrocyte morphology (e.g. poikilocytosis, echinocytosis, etc.) and associated microscopic findings in the bone marrow (erythrocytic hyperplasia), spleen (mild extramedullary hematopoiesis and pigmented macrophages), and liver (increased incidence of Kupffer cells); however, there was lack of a monotonic dose-response, the magnitude of the changes were minimal, and/or were within the variability of the measurements. As a result, the hematological findings were not considered adverse.

Gross findings were limited to the reduced thymus size observed in one male/group at ≥1000 ppm. Differences in organ weights were observed between the 4000 ppm groups and the controls, including increased adrenal gland and spleen weights and reduced thymus, epididymides, testes, prostate, ovary, and uterus with cervix weights. However, these changes primarily lacked a monotonic dose response and/or were similar to controls when using relative to body weight or relative to brain weight metrics indicating the changes in absolute organ weights were an artifact of the reduced absolute body weights at this dose.

There were no corroborating histopathological findings for the adrenal gland, epididymides, testes, prostate, ovary, and uterus. Microscopic generalized lymphoid depletion was observed in the thymus of all animals, including the controls. However, these thymic effects are commonly seen in dogs as part of normal and variable processes of aging that are often observed in dogs as they mature. As a result, they were not considered relevant for human health risk assessment.

The LOAEL was 4000 ppm (equivalent to 114.94 mg/kg/day in males and 131.13 mg/kg/day in females) based on reductions in absolute body weight. The NOAEL was 1000 ppm (equivalent to 26.60 mg/kg bw/day in males and 26.87 mg/kg/day in females).

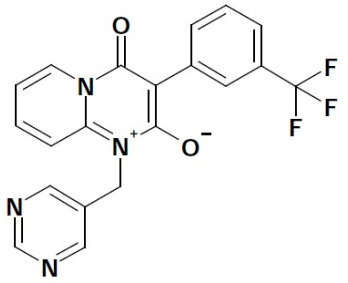
This 90-day oral toxicity study in the dogs is **Acceptable/Guideline** and does satisfy the guideline requirement for a 90-day oral toxicity study (OECD 408) in rats.

COMPLIANCE: Signed and dated GLP, Data Confidentiality, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Triflumezopyrim Technical

Description:	Neutral yellow powder
Batch/Lot #:	RAB55-037
Purity:	99.4 % a.i.
Compound stability:	Stable at room temperature in diet for 15 days
CAS # of TGAI:	1263133-33-0
Structure:	

2. Vehicle: Basal diet

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	Approximately 7.5 to 8.5 months old/weight range: 8.00–11.50 kg for males; 7.10–9.80 kg for females
Source:	Covance Research Products, Inc., Cumberland, Virginia, USA
Housing:	Pair-housed (single sex) in runs with raised flooring, except during the 2-hour feeding period and subsequent clinical observation period, when they were singly housed.
Diet:	Lab Diet® (Certified Canine Diet #5007, PMI Nutrition International, Inc.), 400 g per day. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap Water, *ad libitum*
Environmental conditions:	**Temperature:** 18-29°C **Humidity:** 30-70% **Air changes:** 16-20/hour **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	14 days

B. STUDY DESIGN:

1. In life dates: Start: May 23, 2012; End: August 21, 2012.

2. Animal assignment: Animals were assigned to dose groups by a standard block randomization procedure based on body weights (females) or testes volume (males) as shown in Table 1.

Table 1. Study design[a]					
Test group	Concentration in diet (ppm)	Mean Daily Intake (mg/kg bw/day)		Males	Females
		Male	Female		
Control (1)	0	0	0	4	4
2	100	3.05	2.69	4	4
3	400	12.20	12.15	4	4
4	1000	26.60	26.87	4	4
5	4000	114.94	131.13	4	4

* Data obtained from page 163 of MRID 49382105

3. **Dose selection rationale:** The dietary concentrations were selected based on data from a previous 28-day dog study (MRID 49382159). Five groups of 4 animals/sex/concentration were administered concentrations of triflumezopyrim in feed daily for 90 days, at dietary concentrations of 0, 100, 400, 1000, or 4000 ppm. The untreated or treated canine diet was available once daily (for approximately 2 hours in the morning) for 90 consecutive days. Animals were offered approximately 400 g of meal diet each day. The overall mean daily intake values of DPX-RAB55 for male dogs were 3.05, 12.20, 26.60, or 114.94 mg/kg bw/day, respectively, and for female dogs were 2.69, 12.15, 26.87, or 131.13 mg/kg bw/day, respectively. Due to poor condition in both sexes at 4000 ppm, supplemental feeding of canned food (Hills Prescription Diet A/D) was provided to this group during the last 2 weeks of the study (Days 77 to 90).

4. **Dose preparation and analysis:** The test substance was added to the diet and thoroughly mixed with a Hobart mixer for approximately 10 minutes. The resulting premix was added to additional meal Lab Diet® and was blended for approximately 30 minutes using a twin shell blender. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The homogeneity of Triflumezopyrim in the dietary mixtures was checked by analysis at Week -1 and at Weeks 10 and 11. Concentration of triflumezopyrim in the dietary mixtures was checked by analysis at Weeks 1, 6, 11, and 13. Stability was evaluated prior to study start as part of the analytical method validation study. MPI Research study number 125-161 (DuPont-19685-395-1; the method validation study) has established 15-day room temperature stability for the test diet at concentrations of 300-30000 ppm. The 15-day stability for the 100 ppm dose level was subsequently conducted under an extension of the validation study (MPI Research Study Number 125-161). All analyses were conducted using a validated HPLC method.

5. **Results:**

Homogeneity analysis: Pretest homogeneity results found that a blending time of 10 minutes was sufficient for the 1000 and 4000 ppm and 20 minutes for 400 ppm. However, it was determined that a blending time of 30 minutes should be utilized for 100 ppm test diet preparation, because the homogeneity results for 20 minutes remained outside of the relative standard deviation (RSD) specifications (<15%). During the study, the concentration results for the 100 ppm test diet Week 6 were found to be unacceptable (22.6 %RSD). To address the issue, another test batch was prepared using a larger blender size and the homogeneity

analysis showed that this process produced acceptable results (8.4 %RSD). The preparation process was changed for Week 11 and an additional homogeneity evaluation was conducted for the 4000 ppm test diet showing the new process produced acceptable results (1.1 %RSD).

Stability analysis: Confirmed over a period of 15 days at room temperature.

Concentration analysis: Analysis of the test formulation concentration was conducted for Weeks 1, 6, 11, and 13. The findings showed that the mean concentrations for the 100, 400, 1000, or 4000 ppm dose groups ranged 92.5%-103.4%, 96.7%-104.1%, 89.6%-97.8%, or 96.9%-106.5%, respectively, of nominal concentrations. The mean test substance concentrations were almost all ± 10% of the nominal concentrations. During the study, the concentration results for the 100 ppm test diet Week 6 were found to be unacceptable (22.6 %RSD). To address the issue, another test batch was prepared using a larger blender size/volume (larger shell/headspace) and the same 30-minute blending time. This produced acceptable results (101.4% average recovery).

The mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

6. **Statistics:** The group-wise comparisons of data (body weight, body weight gain, food consumption, hematology except leukocyte count, coagulation, clinical chemistry and organ weights) were made using Levene's/ANOVA-Dunnett's/Welch's tests. Levene's test was used to assess homogeneity of group variances for each specified endpoint, for all collection intervals. If Levene's test was not significant ($p>0.01$), a pooled estimate of the variance was computed from a one-way analysis of variance (ANOVA) and utilized by a Dunnett's comparison of each treatment group with the control group. If Levene's test was significant ($p<0.01$), comparisons with the control group were made using Welch's t-test with a Bonferroni correction. For leukocyte counts (total and differential) the log transformed data were analyzed by group-wise comparisons. Food efficiency and urinalysis values (volume, specific gravity and pH) were subjected to rank transformation and analyzed using Dunnett's test. For endpoints that describe categories rather than numerical measures, a test of association between response and treatment using Cochran Mantel Haenszel[8] tests were conducted if there was sufficient variability among the groups. All analyses were done at $p \leq 0.05$.

The reviewer considers the analyses appropriate.

C. METHODS:

1. Observations:

1a. Clinical examinations: Clinical examinations were performed twice daily, inspecting animals for mortality, morbidity, injury, and the availability of food and water. Examinations for detailed clinical observations were conducted once daily (within 1 hour after removal of food).

1b. Neurological evaluations: Neurobehavioral observations were conducted weekly, examining changes in the level of activity, gait, posture, altered strength, and response to handling as well as the presence of clonic or tonic movements, stereotypies or bizarre behaviour (*e.g.*, self-mutilation).

2. Body weight: Animals were weighed on study day 1 (prior to dosing), weekly after the initiation of dosing, and at the terminal sacrifice.

3. Food consumption/Food efficiency: Food consumption was recorded daily for each animal, and the mean group food consumption was calculated over the weekly weighing interval, as well as monthly and overall. Food efficiency and daily intake of the test article were calculated from food consumption and body weight data.

4. Ophthalmoscopic examination: All animals were examined by focal illumination and indirect ophthalmoscopy prior to the beginning of treatment and again prior to terminal sacrifice.

5. Hematology and clinical chemistry: Blood and urine samples were collected from all animals once during the pretest, during Weeks 4 and 8, and prior to the terminal sacrifice. Animals were fasted overnight prior to sample collection. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscle. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscle. volume (MCV)*
X	Platelet count*	X	Reticulocyte count (Absolute)
	Blood clotting measurements*	X	Blood cell morphology
X	(Thromboplastin time [APTT])		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Globulin*
	Magnesium	X	Creatinine*
X	Inorganic phosphorus	X	Urea nitrogen*
X	Potassium*	X	Total Cholesterol*
X	Sodium*	X	Albumin/Globulin ratio
	ENZYMES (more than 2 hepatic enzymes eg. *)	X	Glucose*
X	Alkaline phosphatase (ALK/also ALP))*	X	Total bilirubin
	Cholinesterase (ChE)	X	Total protein (TP)*
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase (LDH)	X	Bile acid
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. Urinalysis: The CHECKED (X) parameters were examined.

X	Appearance (color)*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for subchronic oral non- rodent studies

7. Sacrifice and pathology: All dogs were euthanized by anesthesia with sodium pentobarbital. Gross pathological examinations were performed on all animals. The CHECKED (X) tissues collected from animals of all the dose groups and preserved in buffered 10% formalin except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson's fixative prior to placing them in formalin. A full complement of tissues and organs was collected from all animals. The CHECKED (X) tissues from animals of the treated and control groups were sectioned, stained with H&E and subjected to histopathological examination. The (XX) organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC. /HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*-Sciatic
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*(3 levels)	X	Lymph nodes*(Mandibular	X	Pituitary*
X	Duodenum*		and Mesenteric)	X	Eyes (optic nerve)*
X	Jejunum*	XX	Spleen*+	X	**GLANDULAR**
X	Ileum*	XX	Thymus*+	XX	Adrenal gland*+
X	Cecum*	X	**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*
X	Rectum*	X	Urinary bladder*/Ureters	XX	Thyroid
XX	Liver*+	XX	Testes*+		Coagulating glands
XX	Gall bladder (not rat)*	XX	Epididymides*+	X	Mammary gland*
X	GALT[a]	X	Prostate*	X	OTHERS
X	Pancreas*	X	Seminal vesicles*	X	Skin*
X	**RESPIRATORY**	XX	Ovaries*+	X	All gross lesions and tissue masses*
X	Trachea*	XX	Oviducts	X	Target organs
X	Lung*	XX	Uterus*+	X	Skeletal muscles
X	Nose*	X	Cervix	X	Sternum/femur
X	Larynx*/Pharynx			X	Tibiofemoral joint

* Recommended for 90-day oral rodent studies based on Guideline 870.3150
aGut associated lymphoid tissue

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Treatment-related clinical observations included thin appearance in 2/4 males and 3/4 females of the 4000 ppm group. A few other clinical signs were observed sporadically during 90-days of treatment in the 4000 and/or 1000 ppm groups and were considered treatment-related, including inappetence (1/4 females at 4000 ppm and 1/4 males at ≥1000 ppm) and decreased activity (2/4 males at 4000 ppm and 2/4 females at 4000 ppm). The female animal at 4000 ppm that was euthanized *in extremis* on study day 77 exhibited inappetence, decreased activity, rapid breathing, loss of skin elasticity, prostration, cold skin, and thin appearance prior to its euthanasia. Other observations noted during the study are either common in this species or occurred at a lower incidence, and therefore, were not considered related to the treatment-related or biologically relevant.

2. **Neurological Observations:** There were no toxicologically relevant changes in neurobehavioral parameters.

B. BODY WEIGHT AND WEIGHT GAIN:

Mean body weights and body weight gains are shown in Table 2. There was a treatment-related decrease (compared to control) in absolute mean body weight and body weight gain observed in both sexes at 1000 and 4000 ppm. For the 1000 ppm dose group absolute body

weight was decreased 10.4% and 16% in males and females, respectively. In the 4000 ppm dose group absolute bodyweight was decreased 23% and 19% in males and females, respectively. At the 4000 ppm group, these findings were supported by statistically significant decreases in body weight gain.

Table 2. Average body weights of dogs during 90 days of treatment[a]

Dose (ppm)	Body weight (kg ± SD)				Cumulative body weight gain (kg)
	Day 1	Day 29	Day 57	Day 91	
Males					
0	10.163 ± 0.45	10.425 ± 1.04	11.075 ± 0.64	10.688 ± 0.51 [b]	0.525
100	9.875 ± 1.54	10.263 ± 1.64	10.313 ± 1.66	9.625 ± 1.89 **(-9.9)**	-0.250
400	10.213 ± 0.89	10.525 ± 1.15	10.463 ± 1.01	9.913 ± 1.20 **(-7.3)**	-0.300
1000	9.800 ± 1.17	9.975 ± 1.35	10.288 ± 1.42	9.575 ± 1.44 **(-10.4)**	-0.225
4000	9.475 ± 0.93	9.375 ± 0.98	9.275 ± 0.89	8.225± 0.94 **(-23.0)**	-1.250**
Females					
0	8.100 ± 0.87	8.550 ± 1.17	8.813 ± 1.35	8.575 ± 1.27	0.475
100	8.100 ± 0.78	8.613 ± 1.15	9.088 ± 1.49	8.313 ± 1.21 **(-3.1)**	0.213
400	8.375 ± 1.10	8.675 ± 1.38	9.225 ± 1.41	8.613 ± 1.65 **(+0.4)**	0.238
1000	7.713 ± 1.03	7.825 ± 0.99	7.863 ± 0.90	7.200 ± 0.60 **(-16.0)**	-0.513
4000	8.238 ± 1.16	8.175 ± 1.38	7.900 ± 1.30	6.950 ± 1.26 **(-19.0)**	-1.000**

[a] Data from pages 31 and 32 of MRID 49382163
Values in parentheses indicate % difference from control calculated by Reviewer.
** Significantly different from control ($p \leq 0.01$).

C. FOOD CONSUMPTION/FOOD EFFICIENCY:

Food consumption and food efficiency data are shown in Table 3. Reductions (compared to control) in food consumption and food efficiency were observed in both sexes at 4000 and 1000 ppm (variable statistical significance). Males and females fed the diets containing 1000 and 4000 ppm triflumezopyrim had lower food consumption compared to controls (11.0% for males and 17.7% for females at 1000 ppm; 22.8% for males and 7.2% for females at 4000 ppm). Decreases in food efficiency at 1000 ppm were minimal (1.1-2.6%), while larger decreases in food efficiency were observed at 4000 ppm (5.8% in males and 5.2% in females).

Table 3. Mean Food consumption/food efficiency in dogs during 90 days of treatment[a]

Parameter	0 ppm	100 ppm	400 ppm	1000 ppm	4000 ppm
Males					
Food consumption (g/animal/day), Weeks 1-13	304.19 - -	314.79 (+3.5%)[b]	318.27 (+4.6%)	270.88 (-11.0%)	234.85 (-22.8%)
Food efficiency (%), Weeks 1–13	1.85	-0.79	-1.19	-1.11	-5.84*
Females					
Food consumption (g/animal/day), Weeks 1-13	247.21 - -	238.82 (-3.6 %)	271.88 (+9.5 %)	203.47 (-17.7 %)	229.52 (-7.2 %)
Food efficiency (%), Weeks 1–13	1.91	0.85	0.77	-2.55	-5.28**

[a] Data from pages 33 and 34 of MRID 49382163
[b] Values in parenthesis indicate % increase or decrease compared to control; calculated by the reviewer
*Significantly different from control by the Levene's criteria, $p < 0.05$.
**Significantly different from control by the Levene's criteria, $p < 0.01$.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related effects were found.

E. BLOOD ANALYSES:

Hematology: Hematological findings are presented in Table 4. At Week 8, there was a decrease of 8-12% in red cell mass (erythrocytes, hemoglobin, and hematocrit) for both sexes at 4000 ppm relative to the control groups. At Week 13, the decrease in red cell mass was greater (↓19-22% relative to the controls) with the difference from the controls for most of the parameters being statistically significant. Reticulocyte responses were not observed in males, but were seen in females at Week 13. These changes were occasionally associated with mild effects on erythrocyte morphology (e.g. poikilocytosis, echinocytosis, etc.) that were suggestive of erythrocyte membrane injury and hemolysis. Overall, the hematological findings tended to lack a monotonic dose-response, the magnitude of the changes were minimal, and/or were within the variability of the measurements; therefore, these effects were not considered adverse.

There were no treatment-related differences in coagulation parameters in male or female dogs. The female that was euthanized on Day 77 had mildly prolonged activated partial thromboplastin time (APTT). This finding correlates with gross and microscopic hemorrhage or may be related to the moribund state of the animal and not treatment with the test substance.

Table 4. Hematology findings in 90-Day feeding study in dogs[a]

Parameter	0 ppm	100 ppm	400 ppm	1000 ppm	4000 ppm
Males (Week 8)					
RBC[a] × 10^6/µL	6.713±0.58	6.913±0.72	6.715±0.61	6.950±0.02	6.170±0.33 (-8%)
Hb[b] (g/dL)	15.13±1.56	14.85±1.34	14.48±1.18	15.00±0.52	13.58±0.77 (-10%)
HCT[c] (% control)	45.15±3.68	45.58±4.01	43.90±3.68	45.35±1.26	41.48±2.19 (-8%)
Males (Week 13)					
RBC × 10^6/µL	6.855±0.72	6.735±0.82	6.433±0.57	6.998±0.10	5.470±0.68* (-20%)
Hb (g/dL)	15.53±1.30	14.73±1.58	13.95±1.24	15.08±0.62	12.25±1.61* (-21%)
HCT (% control)	46.20±3.23	44.15±4.78	41.75±3.61	45.23±1.80	37.33±4.30* (-19%)
Females (Week 8)					
RBC × 10^6/µL	6.763±0.75	6.700±0.41	6.995±0.12	6.658±0.36	6.165±0.48 (-9%)
Hb (g/dL)	14.78±1.62	14.83±1.05	15.45±0.55	14.73±0.54	13.15±0.93 (-11%)
HCT (% control)	45.33±4.96	44.98±3.18	47.03±1.18	45.20±1.22	40.03±2.89 (-12%)
Females (Week 13)					
RBC × 10^6/µL	6.395±0.60	6.540±0.38	6.650±0.28	6.503±0.33	5.120±1.46 (-20%)
Hb (g/dL)	14.23±1.45	14.68±0.81	14.88±0.74	14.68±0.62	11.10±2.82* (-22%)
HCT (% control)	42.90±3.92	44.08±2.21	44.45±1.93	44.25±1.21	34.53±7.62* (-20%)

[a]Data from pages 270, 271, 278 and 279 of MRID 49382163
*Significantly different from control, $p < 0.05$.

Clinical chemistry: Selected clinical findings are summarized in Table 5. There were no treatment-related effects observed. Any differences from the control primarily lacked a monotonic dose response, were of small magnitude, and/or were within the variability of the measurement.

Table 5. Selected clinical chemistry parameters in dogs administered Triflumezopyrim for 90 days					
Parameter	Dose				
	0 ppm	100 ppm	400 ppm	1000 ppm	4000 ppm
Males (Week 8)					
Albumin (g/dL)	2.93	2.98	2.90	2.98	2.68
Calcium (mg/dL)	9.63	9.65	9.60	9.85	9.48
Globulin (g/dL)	2.68	2.85	3.08	2.85	2.83
Alkaline phosphatase (U/L)	47.8	40.8	58.8	49.3	46.0
Males (Week 13)					
Albumin (g/dL)	2.95	2.95	2.90	3.00	2.33**
Calcium (mg/dL)	9.88	9.70	9.90	10.05	9.30**
Globulin (g/dL)	2.20	2.43	2.70	2.30	2.93
Alkaline phosphatase (U/L)	39.3	35.0	56.5	45.0	84.8 (116%)
Females (Week 8)					
Albumin (g/dL)	3.03	3.03	3.08	2.90	2.58*
Calcium (mg/dL)	9.83	9.83	9.95	9.68	9.48
Globulin (g/dL)	2.73	2.80	2.68	2.88	3.05
Alkaline phosphatase (U/L)	51.0	45.0	58.5	39.8	75.5
Females (Week 13)					
Albumin (g/dL)	2.90	3.05	2.95	2.85	2.33*
Calcium (mg/dL)	9.93	9.80	10.08	9.73	9.80
Globulin (g/dL)	2.38	2.38	2.43	2.45	3.33*
Alkaline phosphatase (U/L)	50.0	42.5	61.5	37.8	91.7*(83%)

[a] Data from pages 294-297, 302, 303, 306-309, 314 and 315 of MRID 49382163

*Significantly different from control, $p < 0.05$; **Significantly different from control, $p < 0.01$

F. URINALYSIS: There were no treatment-related differences in urinalysis parameters in either sex.

G. SACRIFICE AND PATHOLOGY:

Organ weight: At 4000 ppm, differences were observed in the weight of some organs (Table 6). These organs included adrenal gland (males), thymus (males and females), spleen, and a number of reproductive organs. These changes primarily lacked a monotonic dose response and/or were similar to controls when using relative to body weight or relative to brain weight metrics indicating the changes were an artifact of the reduced absolute body weights at this dose. The effects on reproductive organs may also be attributed to the effects of malnutrition on sexual maturation. Thymus weights were also reduced in 1000 ppm males and females.

p

Table 6. Absolute and relative organ weights in dogs administered Trifluzomezopyrim for 90 days.[a,b]					
Parameter	Dose				
	0 ppm	100 ppm	400 ppm	1000 ppm	4000 ppm
Male					
Adrenal Gland					
Absolute organ weight (g)	0.988	1.055	1.022	1.094	1.246* (+26%)
Relative to body weight (%)	0.0093	0.0113	0.0103	0.0115	0.0151** (+62%)
Relative to brain weight (ratio)	0.0123	0.0142	0.0131	0.0152	0.0171** (+39%)
Thymus					
Absolute organ weight (g)	9.717	6.183	6.986	5.737	4.223**(-57%)
Relative to body weight (%)	0.0909	0.0645	0.0709	0.0626	0.0508 (-44%)

Relative to brain weight (ratio)	0.1211	0.0828	0.0909	0.0798	0.0587* (-51%)
Epididymides					
Absolute organ weight (g)	3.435	3.040	3.286	3.036	2.606 (-24%)
Relative to body weight (%)	0.0320	0.0324	0.0332	0.0318	0.0315 (-1.6%)
Relative to brain weight (ratio)	0.0430	0.0410	0.0422	0.0416	0.0356 (-17%)
Testes					
Absolute organ weight (g)	15.616	14.505	13.216	14.513	12.213 (-22%)
Relative to body weight (%)	0.1460	0.1534	0.1328	0.1533	0.1472 (+0.82%)
Relative to brain weight (ratio)	0.1949	0.1947	0.1693	0.2022	0.1665 (-15%)
Prostate					
Absolute organ weight (g)	5.360	6.031	5.284	4.941	2.660 (-50%)
Relative to body weight (%)	0.0496	0.0636	0.0550	0.0497	0.0322 (-35%)
Relative to brain weight (ratio)	0.0670	0.0813	0.0694	0.0660	0.0370 (-44%)
Spleen					
Absolute organ weight (g)	58.783	47.222	45.182	61.068	48.032 (-18%)
Relative to body weight (%)	0.5429	0.5118	0.4707	0.6506	0.5788 (+6.6%)
Relative to brain weight (ratio)	0.7409	0.6391	0.5933	0.8544	0.6589 (+89%)
Females					
Adrenal Gland					
Absolute organ weight (g)	1.066	1.057	1.168	0.933	1.132 (+6.2%)
Relative to body weight (%)	0.0127	0.0129	0.0136	0.0130	0.0167 (+31%)
Relative to brain weight (ratio)	0.0145	0.0146	0.0158	0.0129	0.0154 (+6.2%)
Thymus					
Absolute organ weight (g)	7.969	7.021	8.477	5.664	3.631 (-54%)
Relative to body weight (%)	0.0897	0.0837	0.0983	0.0799	0.0527 (-41%)
Relative to brain weight (ratio)	0.1040	0.0959	0.1144	0.0793	0.0494 (-53%)
Ovaries with oviducts					
Absolute organ weight (g)	2.651	1.828	1.825	1.818	1.134* (-57%)
Relative to body weight (%)	0.0312	0.0225	0.0215	0.0254	0.0168 (-46%)
Relative to brain weight (ratio)	0.0355	0.0255	0.0246	0.0249	0.0152* (-57%)
Uterus with cervix					
Absolute organ weight (g)	14.851	6.873	8.905	9.576	2.734 (-82%)
Relative to body weight (%)	0.1732	0.0855	0.1011	0.1319	0.0399 (-77%)
Relative to brain weight (ratio)	0.1983	0.0964	0.1165	0.1301	0.0361 (-82%)
Spleen					
Absolute organ weight (g)	59.256	51.970	51.890	42.360	92.135 (+55%)
Relative to body weight (%)	0.7206	0.6086	0.6199	0.5977	1.3875 (+93%)
Relative to brain weight (ratio)	0.8222	0.6980	0.7112	0.5888	1.3005 (+201%)

[a] Data from pages 39 and 40 of MRID 49382163

[b]Relative weight is defined as the organ to body weight ratio.

* $p \leq 0.05$; ** $p \leq 0.01$

2. <u>Gross pathology</u>: The gross findings were limited to reduced thymus size in one male/group at ≥1000 ppm.

3. Microscopic pathology: Selected microscopic findings are summarized in Table 7. Microscopic generalized lymphoid depletion was observed in the thymus of all animals, including the controls. This finding is commonly observed in dogs as they mature.

At 4000 ppm, microscopic findings in the bone marrow (erythrocytic hyperplasia), spleen (mild extramedullary hematopoiesis and pigmented macrophages), and liver (increased incidence of Kupffer cells) were observed.

Reproductive tract immaturity was also noted in some animals at 4000 ppm; however, onset of sexual maturity may be highly variable and these observations were considered reflective of individual variation in the stage of sexual maturity and not directly related to test substance administration.

Table 7. Selected microscopic findings[a]

Dose level: ppm	0 ppm		100 ppm		400 ppm		1000 ppm		4000 ppm	
Sex (4/sex/group)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
LIVER: Pigment, increased kupffer cell	0	0	0	0	0	0	0	0	3	4
- minimal	0	0	0	0	0	0	0	0	0	1
- mild	0	0	0	0	0	0	0	0	2	1
- moderate	0	0	0	0	0	0	0	0	1	2
Hematopoiesis, extramedullary (EMH)	1	0	0	1	0	1	0	0	0	1
- minimal	1	0	0	1	0	1	0	0	0	0
- mild	0	0	0	0	0	0	0	0	0	1
SPLEEN: Hematopoiesis, extramedullary, increased EMH)- mild	0	0	0	0	0	0	0	0	1	2
Macrophages, pigmented, increased	0	0	1	2	0	2	1	1	3	1
- minimal	0	0	1	2	0	2	1	1	0	1
- mild	0	0	0	0	0	0	0	0	2	0
- moderate	0	0	0	0	0	0	0	0	1	0
BONE MARROW, FEMUR Hyperplasia, erythrocytic	0	0	0	0	0	0	1	0	3	3
- minimal	0	0	0	0	0	0	1	0	3	0
- mild	0	0	0	0	0	0	0	0	0	3
BONE MARROW, RIB Hyperplasia, erythrocytic	0	0	0	0	0	0	1	0	4	3
- minimal	0	0	0	0	0	0	1	0	0	0
- mild	0	0	0	0	0	0	0	0	4	3
BONE MARROW, STERNUM Hyperplasia, erythrocytic- minimal	0	0	0	0	0	0	1	0	3	3
THYMUS: Depletion, lymphoid, generalized	4	4	4	4	4	4	4	4	4	4
- minimal	4	4	4	3	3	4	2	3	0	0
- mild	0	0	0	1	1	0	2	0	1	2
- moderate	0	0	0	0	0	0	0	1	3	2

[a] Data from pages 43, 540, 582-585, 588-591, 600-603, 606-609, 612-615, 620-629 of MRID 49382163;
* $p \leq 0.05$; ** $p \leq 0.01$
M – Male; F – Female

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study authors concluded that dietary administration of Triflumezopyrim to Beagle dogs over 90 days produced treatment-related effects in ≥ 1000 ppm dose males and females. No effects were observed in 400 and 100 ppm males and females. Treatment-related reduction in body weight and nutritional parameters were observed in males at 4000 ppm and in females at ≥1000 ppm compared to controls.

At 4000 ppm, there were decreases in red cell mass, changes to erythrocyte morphology and associated microscopic findings in the bone marrow, spleen, and liver, possibly caused by hemolysis. Increased spleen weights in females at 4000 ppm were secondary to hematological effects observed in this group. Mild decreases in albumin and calcium, and increases in globulin at 4000 ppm indicated a mild inflammatory pattern but these findings were not supported by histopathology.

The changes considered secondary to reduced body weight and food intake, and systemic stress included: 1) organ weight changes at 4000 ppm such as increased adrenal gland weights (males) and reduced thymus (both sexes at ≥1000 ppm), epididymides, testes, prostate, ovary, and uterus with cervix weights; 2) gross findings including small size thymus with corresponding increased severity of microscopic generalized lymphoid depletion in both sexes at ≥1000 ppm; and 3) microscopic changes in the adrenal glands and lymphoid system in both sexes at 4000 ppm, increased severity of generalized lymphoid depletion of the thymus in both sexes at ≥1000 ppm and immaturity of the reproductive tract in both sexes at 4000 ppm.

Under the conditions of this study, the NOAEL for DPX-RAB55 was 400 ppm in male and female dogs. In both males and females, the NOAEL is based on reduced thymic weights with increased severity of thymic lymphoid depletion at >1000 ppm. In females, the NOAEL is also based on reductions in body weight and nutritional parameters at >1000 ppm. This NOAEL is equivalent to 12.20 and 12.15 mg/kg bw/day in males and females, respectively. The effects at 4000 ppm are considered to have exceeded a maximum tolerated dose for both sexes.

B. REVIEWERS' COMMENTS:

Treatment-related decreases in body weight and body weight gain were noted in males at 4000 ppm and in females at ≥1000 ppm, when compared to controls. Reductions in food consumption and/or food efficiency were also noted at these doses; however, decreases in food efficiency at 1000 ppm were minimal (1-3%). In a chronic study in dogs (MRID 49382164) doses up to 53/56 mg/kg/day (M/F) did not produce any changes in absolute body weight. Furthermore, in a range-finding 28-day oral study in dogs (MRID 49382159) that tested two dogs/dose, decreases in absolute body weights were only seen at doses > 55 mg/kg/day. In the current study, although reductions in absolute body weight were ≥10% at

1000 ppm, they were not considered adverse based on the weight of evidence across the available oral toxicity studies in dogs.

Mild changes in red blood cell parameters were noted at 4000 ppm. Alterations in erythrocyte morphology and associated microscopic findings (particularly in the bone marrow and spleen) were also observed that may be a response to erythrocyte hemolysis. Due to the lack of dose response, minimal magnitude of the changes, and/or variability of the measurements, the hematological findings were not considered adverse.

Differences in organ weights were observed between the 4000 ppm groups and the controls, including increased adrenal gland weights and reduced thymus, epididymides, testes, prostate, ovary, and uterus with cervix weights. However, these changes primarily lacked a monotonic dose response and/or were similar to controls when using relative to body weight or relative to brain weight metrics indicating the changes were an artifact of the reduced absolute body weights at this dose. Furthermore, there were no corroborating histopathological findings for the adrenal gland, epididymides, testes, prostate, ovary, and uterus. For microscopic findings, generalized lymphoid depletion was observed in the thymus of all animals, including the controls. These thymic effects are commonly seen in dogs as part of normal and variable processes of aging and are often observed in dogs as they mature[2]. As a result, the thymic effects were not considered relevant for human health risk assessment.

The LOAEL was 4000 ppm (equivalent to 114.94 mg/kg/day in males and 131.13 mg/kg/day in females) based on reductions in absolute body weight. The NOAEL was 1000 ppm (equivalent to 26.60 mg/kg bw/day in males, 26.87 mg/kg/day in females).

B. STUDY DEFICIENCIES:

None

[2] Sellers *et al.* (2007). Society of toxicologic pathology position paper: Organ weight recommendations for toxicology studies [Review]. Toxicol Pathol 35: 751-755.
Sato *et al.* (2012). Histopathology of incidental findings in Beagles used in toxicity studies. J. Toxicol. Pathol. 25:103-134.
Michael *et al.* (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices [Review]. Toxicol Pathol 35: 742-750.